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AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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E11	1	LUKYANOVA C N/AU
E12	3	LUKYANOVA E/AU

=> s cnidarian and (anthozoan)

L1 123 CNIDARIAN AND (ANTHOZOAN)

=> s 12 and (nucleic acid0 UNMATCHED LEFT PARENTHESIS 'AND (NUCLEIC' The number of right parentheses in a query must be equal to the number of left parentheses.

=> s 12 and nucleic acid

L2 NOT FOUND

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=> s 11 and nucleic acid

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SEARCH ENDED BY USER 4 FILES SEARCHED...

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(FILE 'HOME' ENTERED AT 11:08:06 ON 22 SEP 2006)

FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCAPLUS, WPIDS, FSTA, JICST-EPLUS, SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006

E LUKYANOV, S/AU

123 S CNIDARIAN AND (ANTHOZOAN) L1

=> s l1 and (non-bioluminescent)

L2 11 L1 AND (NON-BIOLUMINESCENT)

=> s l1 and (non-pennatulacean)

5 L1 AND (NON-PENNATULACEAN) L3

=> d l3 ti abs ibib tot

ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN L3 ·

Rapidly maturing fluorescent proteins and methods for using the same TI

Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as AB well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-

Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. red fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

2005:588435 HCAPLUS ACCESSION NUMBER:

DOCUMENT NUMBER:

143:112117

TITLE:

Rapidly maturing fluorescent proteins and methods for

using the same

INVENTOR(S): PATENT ASSIGNEE(S): Bevis, Brooke; Glick, Benjamin

The University of Chicago, USA SOURCE:

U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of Appl.

No. PCT/US02/40539.

CODEN: USXXCO

DOCUMENT TYPE:

Patent English

LANGUAGE: FAMILY ACC. NUM. COUNT:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005149994	A1	2005070.7	US 2004-844064	20040511
WO 2003054158	A2	20030703	WO 2002-US40539	20021218
WO 2003054158	A3	20031204		•
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            PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,
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                                           US 2001-341723P P 20011219
PRIORITY APPLN. INFO.:
                                           WO 2002-US40539 A2 20021218
    ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN
L3
    Novel Cnidarian and Anthozoan chromo/fluoroproteins
TI
     and cDNA encoding them
```

DNA encoding novel chromo/fluoroproteins as well as the encoded proteins AB are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian , e.g., Anthozoan , species or are obtained from Anthozoan non- Pennatulacean (sea pen) Specific proteins of interest include the following specific proteins: Heteractis crispa hcriGFP; Dendronephthya dendGFP; Zoanthus zoanRFP; Scolymia cubensis scubGFP1 and scubGFP2; Ricordea florida rfloRFP, rfloGFP2, and rfloGFP; Montastraea cavernosa mcavRFP, mcavGFP, and mcavGFP2; Condylactis gigantea cgigGFP; Agaricia fragilis afraGFP; and Montastraea annularis mannFP. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER:

2005:122690 HCAPLUS

DOCUMENT NUMBER:

INVENTOR(S):

142:193054

TITLE:

Novel Cnidarian and Anthozoan

chromo/fluoroproteins and cDNA encoding them
Labas, Yulii Aleksandrovich; Gurskaya, Nadezda
Georgievna; Yanushevich, Yuriy; Fradkov, Arcady
Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;

Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S):

SOURCE:

Russia

U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of Appl. No. PCT/02US/36499.

CODEN: USXXCO

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT:

PATENT NO.	KIND DATE	E APPLIO	CATION NO.	DATE
US 2005032085	A1 2005	50210 US 200	04-757356	20040113
WO 2003042401	A2 2003	30522 WO 200	02-US36499	20021112
WO 2003042401	A3 2003	31120		
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GM, HR, HU,	ID, IL, IN,	, IS, JP, KE, I	KG, KP, KR, KZ,	LC, LK, LR,
LS, LT, LU,	LV, MA, MD,	, MG, MK, MN, N	MW, MX, MZ, NO,	NZ, OM, PH,
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PRIORITY APPLN. INFO.:

US 2001-332980P P 20011113

WO 2002-US36499 A2 20021112

L3 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN

TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes

AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-

Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. Claimed sequences were not present at the time of publication.

ACCESSION NUMBER: 2003:511470 HCAPLUS

DOCUMENT NUMBER: 139:65739

TITLE: Engineering rapidly maturing variants of the Discosoma

red fluorescent protein (DsRed) and their use as

bioluminescent probes

INVENTOR(S): Bevis, Brooke; Glick, Benjamin PATENT ASSIGNEE(S): The University of Chicago, USA

SOURCE: PCT Int. Appl., 65 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

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EP	1456	223			A2		2004	0915	1	EP 20	002-8	80562	20		20	00212	218
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US 2005149994 A1 20050707 US 2004-844064 20040511
PRIORITY APPLN. INFO.: US 2001-341723P P 20011219
WO 2002-US40539 W 20021218

L3 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN

TI cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use

Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants AB thereof, as well as the proteins encoded the same, are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. More specifically, they include GFP of Heteractis crispa, Dendronephthya sp, Scolymia cubensis, Ricordea florida, Montastraea cavernosa, Condylactis gigantea, Agaricia fragilis, sequence homolog of Montrastraea annularis and RFP of Zoanthus sp., Ricordea florida, and Montastraea cavernosa. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2003:397030 HCAPLUS

DOCUMENT NUMBER: 138:397335

TITLE: cDNAs encoding chromo/fluoroproteins from

non-bioluminescent Cnidarian species or

non-Pennatulacean (sea pen) species

and their use

INVENTOR(S): Labas, Yulii Aleksandrovich; Gurskaya, Nadezda

Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;

Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA

SOURCE: PCT Int. Appl., 88 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PAT	TENT	NO.			KINI)	DATE		j	APPL:	I CAT	ION 1	. O <i>l</i> .			ATE	
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WO	2003	0424	01		A2		2003	0522	Ĭ	WO 2	002-1	JS36	499		2	0021	112
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JP 2005509420 T2 20050414 JP 2003-544215 20021112 US 2005032085 A1 20050210 US 2004-757356 20040113 PRIORITY APPLN. INFO.: US 2001-332980P P 20011113 WO 2002-US36499 W 20021112

L3 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN

TI cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same

Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants AB thereof, as well as the proteins encoded by the same, are provided. subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian , e.g., Anthozoan, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific Anthozoan species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp. (NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red" (NFP-6), Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp. "magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2002:978391 HCAPLUS

DOCUMENT NUMBER:

138:50935

TITLE:

cDNA and protein sequences of novel

chromo/fluoroproteins from non-bioluminescent

Cnidarian species or are obtained from non-Pennatulacean (sea pen) species

and methods for using the same

INVENTOR(S):

Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii

A.; Matz, Mikhail V.; Terskikh, Alexey

PATENT ASSIGNEE(S):

Russia

SOURCE:

U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of Appl.

No. PCT/US00/28477.

CODEN: USXXCO

DOCUMENT TYPE: LANGUAGE: Patent English

FAMILY ACC. NUM. COUNT:

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FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCAPLUS, WPIDS, FSTA, JICST-EPLUS, SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006

E LUKYANOV, S/AU

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ANSWER 1 OF 11 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents; involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic animal construction 2003-22525 BIOTECHDS

AN 2003-22525 BIOTE AB DERWENT ABSTRACT:

NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromoor fluorescent protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment of a nucleic acid encoding (I); (2) a construct comprising a vector and a nucleic acid encoding (I); (3) an expression cassette (II) comprises, a transcriptional initiation region that is functional in an expression host, a nucleic acid encoding (I) and a transcriptional termination region functional in the expression host; (4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell; (5) producing a chromo and/or fluorescent protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins; (6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III); (7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I); (8) a kit comprising a nucleic acid encoding (I); (9) preparation (M1) of nucleic acid encoding (I); and (10) a nucleic acid produced by (M1).

BIOTECHNOLOGY - Preparation: Producing a nucleic acid that encodes a protein having at least one point mutation chosen from positions 148 and 165 as compared to the parent protein produced by the nucleic acid encoding (I). The produced nucleic acid encodes a protein having point mutations at both positions 148 and 165. (I) is the fluorescent mutant of parent non-fluorescent chromoprotein. The nucleic acid produced encodes a protein further comprising mutations at positions 167 and 203, where (I) is a non-fluorescent chromoprotein of a parent fluorescent protein (claimed). Preferred Nucleic Acid: The chromo or fluorescent protein is from a non-bioluminescent Chidarian sp and belongs to Anthozoan sp.. (I) includes a point mutation chosen from a mutation at positions 148 and 165. (I) is a fluorescent mutant of a chromoprotein and includes a point mutation at both positions 148 and 165. (I) is a non-fluorescent chromoprotein of a fluorescent protein and includes a point mutation at positions 148 and 165. (I) a non-fluorescent chromoprotein of a fluorescent protein and includes a point mutation at positions 167 and 203.

USE - Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. Chromoprotein encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The chromoprotein is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca2+ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as

second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

EXAMPLE - A purple chromoprotein, asCP from Anemonia sulcata and a red fluorescent protein DsRed from Discosoma Sp. were selected as representatives of chromoprotein (CP) and fluoroprotein (FP) respectively. Site directed and random mutagenesis were performed to transform CP into FP and vice versa. Site directed mutagenesis was performed by PCR with primers containing target substitution using the overlap extension method. All mutants were cloned into pQE30 vector so that recombinant proteins contained 6-histidine tag at their N-termini. Escherichia coli XL1 Blue cells were transformed with the plasmids according to standard protocols and spread onto 3-4 Petri dishes with LB agar media supplemented with ampicillin for selection. After overnight growth at 37degreesC the plates were stored for 2-5 days at room temperature or 4degreesC to allow proteins to mature completely. The plates were washed with phosphate buffered saline (PBS). Cells were disrupted by sonication and soluble recombinant proteins were purified on the TALON metal affinity resin. Absorption spectra of the proteins were recorded on a Beckman DU520 UV/VIS spectrophotometer. The amino acid substitution in asCP mutant in the positions 148 and 165, increased quantum yield of red fluorescence. Serine and valine were substituted at positions 148 and 165 respectively. In fluorescent DsRed, substitutions at position 148, 165, 167 and 203 significantly decreased fluorescence intensity, and the spectral characteristics of these mutants became more close to those of CPs. Non-fluorescent (NF) mutant DsRed-NF carried four amino acid substitutions, specifically. Ser148Cys, Ile165Asn, Lys167Met and Ser203Ala. DsRed-NF possessed a high extinction coefficient and an extremely low quantum yield (less than 0.001). These special characteristics converted DsRed-NF into a true chromoprotein. (56 pages)

Characteristics converted baked-or into a true thic

ACCESSION NUMBER: 2003-22525 BIOTECHDS

TITLE: Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors,

coloring agents;

involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic

animal construction

AUTHOR: BULINA M E; CHUDAKOV D; LUKYANOV K A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2003057833 17 Jul 2003 APPLICATION INFO: WO 2002-US41418 23 Dec 2002

PRIORITY INFO: US 2001-343128 26 Dec 2001; US 2001-343128 26 Dec 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-607998 [57]

ANSWER 2 OF 11 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent
mutant of a Chidarian chromo- or fluorescent protein or its
mutant, useful for applications involving chromo- or fluorescent
proteins;

involving vector-mediated gene transfer and expression in Escherichia coli

AN 2003-20948 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or

fluorescent mutant of a Chidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host; (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and (8) a kit comprising (I) or (II).

WIDER DISCLOSURE - (1) homologs of (I); (2) nucleic acids that encode proteins as (I), but differ in sequence of (I) due to degeneracy of genetic code; (3) nucleic acid that hybridize to (I); (4) nucleic acid that encode fusion proteins comprising (VI) and a heterologous protein; (5) homologs or fragments of (VI); and (6) polypeptides that differ from (VI).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) encodes a chromo- or fluorescent mutant of a non-bioluminescent Cnidarian species (e.g. Anthozoan species, preferably Discosoma sp.) chromo- or fluorescent protein. More preferably, (I) encodes a mutant of wild-type Discosoma sp. red fluorescent protein (DsRed), where the nucleic acid encodes a product having a point mutation at at least one of position 2, 5, 6, 21, 41, 42, 44 and 117 relative to wild-type DsRed, preferably a product having a point mutation at at least one of position 145 and 217. The nucleic acid encoding the product has a sequence of residues that is substantially the same or identical to a nucleotide sequence of at least 10 residues in length of a fully defined wild-type DsRed nucleic acid sequence (S1) as given in the specification.

USE - (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed). (I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used is assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

EXAMPLE - Wild-type Discosoma sp. red fluorescent protein (DsRed), an orange-red fluorescence with an emission maximum at 583 nm, had several problems for use as a fluorescent reporter, e.g., slow maturation. Therefore, to identify rapidly maturing DsRed variants, an earlier method for visualizing green fluorescent protein (GFP) fluorescence in microbial colonies was modified. Hexahistidine-tagged DsRed was produced at high levels in Escherichia coli. The fluorescence

of the bacterial colonies was excited by placing a 520 +/- 20 nm bandpass filter over the lens of a slide projector, and the emission was detected. A library of mutant expression plasmids was generated using error-prone PCR to amplify the DsRed1 template. This library was transformed into E. coli, and over 100000 transformant colonies were examined. Colonies producing the wild-type DsRedl protein required two days to develop significant fluorescence, but three mutant colonies showed strong fluorescence after one day of growth. Sequencing revealed that the three mutant plasmids were distinct, but that all of them contained an N42H codon change. Therefore a variant was generated that had only the N42H substitution. The N42H variant was purified in parallel with DsRedl, and the two proteins were analyzed by spectrofluorometry. The spectra of purified DsRedl changed over a period of days as the protein matured. By contrast, the spectra of the purified N42H variant remained stable over time consistent with rapid maturation. In addition to accelerating maturation, the N42H substitution altered the spectral properties of the mature protein. Mature DsRed1 was assumed to be an equilibrium mixture of red fluorescent molecules and some green fluorescent molecules that were spectrally similar to GFP. The GFP-like species had a blue excitation peak at approximately 480 nm and a green emission peak at approximately 500 nm, but DsRed was tetramer, so excitation of the green molecules often resulted in the fluorescence resonance energy transfer (FRET) with neighboring red molecules to produce and emission. The FRET effect, together with the relatively low percentage of green molecules in mature DsRed1, yielded a very small peak of green emission relative to the red emission. In the N42H variant, the peaks of blue excitation and green emission were dramatically enhanced, indicating that the equilibrium had shifted so that a target percentage of the mature molecules contained the green chromophore. Because the N42H substitution considerably increased the size of the side chain, a more conservative N42Q substitution was also tried. This mutation required two base changes and probably would not have been present in the original mutant collection. The N42Q variant retained the rapid maturation property of the N42H variant, but showed much less blue excitation and green emission. The N42Q variant was therefore chosen as the starting point for further study. Additional mutagenesis yielded DsRed variants that showed even faster maturation and lower green emission than the original N42Q variant. After six rounds of mutagenesis, three optimized variants were selected and termed DsRed T1, DsRed T3 and DsRed T4. The spectral properties of DsRed T4 were virtually identical to those of DsRed T1 and very similar to those of the wild-type DsRed1. Compared with DsRed T1 and DsRed T4, DsRed T3 was somewhat brighter but has a significantly higher peak of blue excitation and a marginally higher peak of green emission. The optimized DsRed variants were examined both in vivo and in vitro. As judged by colony fluorescence, colony size, and plasmid stability, these variants were less toxic to E. coli than DsRed1, and they developed fluorescence more efficiently at growth temperatures of 37 degreesC and higher. Like wild-type DsRed, the optimized variants appeared to be tetrameric, they exhibited FRET between the green and red molecules. The end result was pair of optimized variants termed DsRed T3 and DsRed T4. DsRed T4, DsRed T3 matured rapidly, and the purified protein was nearly as bright as mature wild-type DsRed. Making this variant well suited to single-color imaging of red fluorescence. DsRed T3 had a higher peak of blue excitation and a slightly higher peak of green emission than wild-type DsRed. DsRed T4 had fluorescence spectra very similar to those of wild-type DsRed and yielded negligible contamination of the GFP signal. Although purified DsRed T4 was only about half as bright as DsRed T3, this effect was partially offset in vivo because DsRed T4 matures nearly twice as fast as DsRed T3. Thus, DsRed T4 was probably the best variant for most applications. (65 pages)

ACCESSION NUMBER: 2003-20948 BIOTECHDS

TITLE: Novel nucleic acid ence

Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Chidarian chromo- or

fluorescent protein or its mutant, useful for applications

involving chromo- or fluorescent proteins;

involving vector-mediated gene transfer and expression in

Escherichia coli

AUTHOR: BEVIS B; GLICK B

PATENT ASSIGNEE: UNIV CHICAGO

PATENT INFO: WO 2003054158 3 Jul 2003 APPLICATION INFO: WO 2002-US40539 18 Dec 2002

PRIORITY INFO: US 2001-341723 19 Dec 2001; US 2001-341723 19 Dec 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-569236 [53]

ANSWER 3 OF 11 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and

AN 2003-09318 BIOTECHDS

cell sorting

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host; (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I); (8) a transgenic organism (IX) comprising a transgene that comprises (I); (9) production of (VI); (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent; (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

WIDER DISCLOSURE - Also disclosed are: (1) a composition comprising (I), (V), (VI), (VII) or recombinant vectors; (2) homologs of (I) or (VI); (3) nucleic acids that encode the proteins encoded by (I), but differ in sequence from (I) due to the degeneracy of the genetic code; (4) nucleic acids that hybridize to (I) under stringent conditions; (5) nucleic acids encoding variants e.g., mutants of (I); and (6) nucleic acids that encode fusion proteins of (I) or its fragments.

BIOTECHNOLOGY - Preparation: (VI) is produced by growing (V), where (VI) is expressed, and isolating (VI) that is substantially free of other proteins (claimed). Preferred Nucleic Acid: In (I), the kindling stimulus is light of a kindling wavelength, intensity and duration effective to kindle the kindling fluorescent protein. The kindling wavelength of the kindling stimulus ranges from about 200-1500 nm, the kindling intensity of the kindling stimulus ranges from 0.01-106 W/cm2, and the kindling duration of the kindling stimulus ranges from 1 millisecond to about 60 minutes. The kindling fluorescent protein does not have a sequence that is identical to a sequence comprising 232 or 215 amino acids fully defined in the specification. The kindling fluorescent protein is a mutant of a wild type kindling fluorescent protein. The kindling

fluorescent protein is a wild type protein or its mutant from a non-bioluminescent Cnidarian species, preferably an Anthozoan species. The second state is transient. The second fluorescent state is permanent. (I) is an isolated nucleic acid. Preferred Method: In (M), the kindling fluorescent protein is present inside an organism or cell.

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed). (I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

EXAMPLE - Generation of and initial characterization of kindling fluorescent proteins was as follows. Routine target i.e. site specific, and random mutagenesis of wild type asFP595 and Heteractis crispa chromoproteins was carried out. The asFP595 (asCP) had a sequence comprising 232 amino acids fully defined in the specification and was encoded by a nucleotide coding sequence comprising 767 base pairs fully defined in the specification. The H.crispa chromoprotein had a sequence comprising 215 amino acids fully defined in the specification was encoded by a nucleotide coding sequence comprising 760 base pairs fully defined in the specification. Mutagenesis was performed by the overlap extension method. Briefly, two overlapping fragments of each FP coding region were amplified. Polymerase chain reaction (PCR) was carried out using Advantage 2 polymerase mix in 1xmanufacturer's buffer supplemented with 100 muM of each dNTP, 0.2 muM of each primer, and 1 ng of plasmid DNA in 25 mul (final volume). To remove plasmids encoding wild type proteins, the 5' and 3'-fragments were excised from 2% low-melting agarose gel in 1xTAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. For each particular mutant, appropriate 5'- and 3'-fragments were combined to obtain full-length cDNA as follows. Equal volumes of 5'-fragment solution, 3'-fragment solution and 3xPCR mixture containing Advantage 2 polymerase mix, buffer and dNTPs were mixed together and subjected to 2-3 cycles of 95degreesC for 20 s, 65degreesC for 30 min, 72degreesC for 30 s. Then, the reaction was diluted 10 fold and 1 mul of the diluted sample was used as a template for PCR with forward and reverse cloning primers. As a result, ready-for-cloning fragments containing full-length coding regions with target substitution(s) were generated. Mutant PC products were digested with endonucleases, for which the cloning primers contained sites, and then cloned into pQE30, digested with endonucleases generating complementary overhangs. Each of the recombinant proteins generated by both cloning-expression systems contained a 6xHis tag on the N- or C-terminus. Selected Escherichia coli clones were grown at 37degreesC in 50 ml to an optical density of (OD) 0.6. At that point, the expression of recombinant FP was induced with 0.2 mM isopropyl-beta-D-thiogalactoside (IPTG). The cultures were then incubated overnight. The following day, cells were harvested by centrifugation, resuspended in buffer and disrupted by sonication. Fluorescent proteins were purified from the soluble fraction.

Proteins were at least 95% pure according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (96 pages)

ACCESSION NUMBER: 2003-09318 BIOTECHDS

TITLE: Novel nucleic acid that is present in other than its natural

environment and that encodes kindling fluorescent protein, is

useful in labeling protocols, e.g. labeling proteins,

organelles, cells and organisms;

vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer,

high throughput screening and cell sorting

AUTHOR: LUKYANOV S A; CHUDAKOV D; LUKYANOV K

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2002096924 5 Dec 2002 APPLICATION INFO: WO 2002-US16379 24 May 2002

PRIORITY INFO: US 2001-329176 11 Oct 2001; US 2001-293752 25 May 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-156788 [15]

L2 ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN

TI Rapidly maturing fluorescent proteins and methods for using the same

Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan,

species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. red fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject

nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:588435 HCAPLUS

DOCUMENT NUMBER: 143:112117

TITLE: Rapidly maturing fluorescent proteins and methods for

using the same

INVENTOR(S): Bevis, Brooke; Glick, Benjamin PATENT ASSIGNEE(S): The University of Chicago, USA

SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of Appl.

No. PCT/US02/40539.

CODEN: USXXCO

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT NO.	KIND DATE	E APPL	ICATION NO.	DATE
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WO 2003054158	A3 2003	31204		
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RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:

US 2001-341723P

P 20011219

WO 2002-US40539

A2 20021218

- L2 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN
- TI Novel Chidarian and Anthozoan chromo/fluoroproteins and cDNA encoding them

DNA encoding novel chromo/fluoroproteins as well as the encoded proteins AB are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from nonbioluminescent Cnidarian , e.g., Anthozoan , species or are obtained from Anthozoan non- Pennatulacean (sea pen) species. Specific proteins of interest include the following specific proteins: Heteractis crispa hcriGFP; Dendronephthya dendGFP; Zoanthus zoanRFP; Scolymia cubensis scubGFP1 and scubGFP2; Ricordea florida rfloRFP, rfloGFP2, and rfloGFP; Montastraea cavernosa mcavRFP, mcavGFP, and mcavGFP2; Condylactis gigantea cgigGFP; Agaricia fragilis afraGFP; and Montastraea annularis mannFP. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:122690 HCAPLUS

DOCUMENT NUMBER:

INVENTOR(S):

142:193054

TITLE:

Novel Cnidarian and Anthozoan

chromo/fluoroproteins and cDNA encoding them
Labas, Yulii Aleksandrovich; Gurskaya, Nadezda

Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;

Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S):

Russia

SOURCE:

U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of Appl.

No. PCT/02US/36499.

CODEN: USXXCO

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT:

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W :	ΑE,	AG,	AL,	AM,	AT,	AU,	AZ,	BA,	BB,	BG,	BR,	BY,	BZ,	CA,	CH,	CN,
	CO,	CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,
	GM,	HR,	HU,	ID,	IL,	IN,	IS,	JP,	ΚĒ,	KG,	KP,	KR,	KZ,	LC,	LK,	LR,
	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MZ,	NO,	NZ,	OM,	PH,
	PL,	PT,	RO,	RU,	SC,	SD,	SE,	SG,	SI,	SK,	SL,	TJ,	TM,	TN,	TR,	TT,
	TZ,	UA,	UG,	US,	UZ,	VC,	VN,	YU,	ZA,	ZM,	ZW					
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	FI,	FR,	GB,	GR,	IE,	IT,	LU,	MC,	NL,	PT,	SE,	SK,	TR,	BF,	BJ,	CF,

CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.:

WO 2002-US36499

A2 20021112

L2 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN

TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes

Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of

pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. Claimed sequences were not present at the time of publication.

ACCESSION NUMBER: 2003:511470 HCAPLUS

DOCUMENT NUMBER:

139:65739

TITLE:

Engineering rapidly maturing variants of the Discosoma

red fluorescent protein (DsRed) and their use as

bioluminescent probes

INVENTOR(S):
PATENT ASSIGNEE(S):

Bevis, Brooke; Glick, Benjamin The University of Chicago, USA

SOURCE:

PCT Int. Appl., 65 pp.

CODEN: PIXXD2

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT:

PATE	NT I	NO.			KINI	D :	DATE		j	APPL	ICAT:	ION 1	.OV		Di	ATE	
WO 2 WO 2									7	WO 2	002-1	US40!	539		2	0021	218
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		GM,	HR,	HU,	ID,	IL,	IN,	IS,	JP,	KE,	KG,	KP,	KR,	KZ,	LC,	LK,	LR,
		LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MZ,	NO,	NZ,	OM,	PH,
		PL,	PT,	RO,	RU,	SC,	SD,	SE,	SG,	SK,	SL,	TJ,	TM,	TN,	TR,	TT,	TZ,
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CA 2																	
AU 2																	
EP 1																	
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JP 2																	
US 2					A1		2005	0707			004-8						
PRIORITY .	APP]	LN. :	INFO	.:							001-3					00112	219
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ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN L2

cDNAs encoding chromo/fluoroproteins from non-TI

bioluminescent Cnidarian species or non-Pennatulacean

(sea pen) species and their use

Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants AB thereof, as well as the proteins encoded the same, are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Chidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. More specifically, they include GFP of Heteractis crispa, Dendronephthya sp, Scolymia cubensis, Ricordea florida, Montastraea cavernosa, Condylactis gigantea, Agaricia fragilis, sequence homolog of Montrastraea annularis and RFP of Zoanthus sp., Ricordea florida, and Montastraea cavernosa. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2003:397030 HCAPLUS

DOCUMENT NUMBER: 138:397335

cDNAs encoding chromo/fluoroproteins from non TITLE:

-bioluminescent Cnidarian species

or non-Pennatulacean (sea pen) species and their use

Labas, Yulii Aleksandrovich; Gurskaya, Nadezda INVENTOR(S):

Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;

Matz, Mikhail Vladimirovich

Clontech Laboratories, Inc., USA PATENT ASSIGNEE(S):

PCT Int. Appl., 88 pp. SOURCE:

CODEN: PIXXD2 Patent

DOCUMENT TYPE: English LANGUAGE:

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

PA	TENT	NO.			KIN	D	DATE		j	APPL:	ICAT:	ION 1	. 00		D	ATE	
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							DK,										
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ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN L2cDNA and protein sequences of novel chromo/fluoroproteins from non ΤI -bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants AB thereof, as well as the proteins encoded by the same, are provided. The subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Chidarian, e.g., Anthozoan, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific Anthozoan species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp. (NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red" (NFP-6), Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp. "magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. 2002:978391 HCAPLUS

ACCESSION NUMBER:

DOCUMENT NUMBER:

138:50935

TITLE:

cDNA and protein sequences of novel chromo/fluoroproteins from nonbioluminescent Cnidarian species or

are obtained from non-Pennatulacean (sea pen) species

and methods for using the same

INVENTOR(S):

Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii

A.; Matz, Mikhail V.; Terskikh, Alexey

PATENT ASSIGNEE(S):

Russia

SOURCE:

U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of Appl.

No. PCT/US00/28477.

CODEN: USXXCO

DOCUMENT TYPE:

Patent English

LANGUAGE:

17 FAMILY ACC. NUM. COUNT:

PATENT NO.	KIND DATE	APPLICATION NO.	
		US 2001-6922 WO 1999-US29405	
	CY, DE, DK, ES	, FI, FR, GB, GR, IE,	IT, LU, MC, NL,
WO 2001027150	A2 2001041	9 WO 2000-US28477	20001013
WO 2001027150	A3 2001120	5	
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HU, ID, IL,	IN, IS, JP, KI	, KG, KP, KR, KZ, LC,	LK, LR, LS, LT,
LU, LV, MA,	MD, MG, MK, MM	, MW, MX, MZ, NO, NZ,	PL, PT, RO, RU,
SD, SE, SG,	SI, SK, SL, TO	, TM, TR, TT, TZ, UA,	UG, US, UZ, VN,
YU, ZA, ZW			
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DE, DK, ES,	FI, FR, GB, GF	, IE, IT, LU, MC, NL,	PT, SE, BF, BJ,
CF, CG, CI,	CM, GA, GN, GV	, ML, MR, NE, SN, TD,	TG
CA 2434737	AA 2002090	CA 2002-2434737	20020220
WO 2002068459	A2 2002090	6 WO 2002-US5749	20020220
WO 2002068459	A3 2003112	7	

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             GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA,
             GN, GQ, GW, ML, MR, NE, SN, TD, TG
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                                20020912
                                             AU 2002-254031
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                                             US 2002-81864
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    US 2003022287
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                                20051129
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    US 6969597
                                             EP 2002-723238
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                          A2
                                20040204
     EP 1385967
        R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
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    JP 2004536571
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                                             JP 2002-567969
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                                20041209
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                                20030515
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                                                                 A2 19991014
                                             US 1999-418529
PRIORITY APPLN. INFO.:
                                                                  B2 19991015
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                                             US 1999-444338
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                                             US 1999-457898
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                                             WO 2000-US28477
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                                             AU 2001-10867
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                                             US 2001-270983P
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                                             US 2001-293752P
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                                             US 2001-329176P
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                                             US 2001-976673
                                             US 2001-6922
                                                                  A 20011204
                                                                  A1 20020220
                                             US 2002-81864
                                             WO 2002-US5749
                                                                  W 20020220
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- L2 ANSWER 9 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.
- AN 2003-607998 [57] WPIDS
- AB WO2003057833 A UPAB: 20030906

NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromoor fluorescent protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) a fragment of a nucleic acid encoding (I);
- (2) a construct comprising a vector and a nucleic acid encoding (I);
- (3) an expression cassette (II) comprises, a transcriptional initiation region that is functional in an expression host, a nucleic acid encoding (I) and a transcriptional termination region functional in the

expression host;

- (4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell;
- (5) producing a chromo and/or fluorescent protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins;
- (6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III);
- (7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I);
 - (8) a kit comprising a nucleic acid encoding (I);
 - (9) preparation (M1) of nucleic acid encoding (I); and
 - (10) a nucleic acid produced by (M1).

USE - Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. Chromoprotein encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The chromoprotein is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca2+ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

DESCRIPTION OF DRAWING(S) - The figure shows the normalized spectra for selected mutants of asCP and DsRed.

Dwg.3/3

ACCESSION NUMBER: 2003-607998 [57] WPIDS

DOC. NO. CPI: C2003-165725

TITLE: Novel nucleic acid encoding interconverted mutant of

chromo-or fluorescent protein which are useful as

biosensors, coloring agents.

DERWENT CLASS: B04 D16

INVENTOR(S): BULINA, M E; CHUDAKOV, D; LUKYANOV, K A

PATENT ASSIGNEE(S): (CLON-N) CLONTECH LAB INC; (BULI-I) BULINA M E; (CHUD-I)

CHUDAKOV D; (LUKY-I) LUKYANOV K A

COUNTRY COUNT: 103

PATENT INFORMATION:

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU

MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT

RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA

ZM ZW

AU 2002367391 Al 20030724 (200421)

US 2004248180 A1 20041209 (200481)

EP 1504017 A2 20050209 (200512) EN

R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR

JP 2005514032 W 20050519 (200538)

38

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003057833 AU 2002367391	A2 A1	WO 2002-US41418 AU 2002-367391	20021223
US 2004248180	Al Provisional CIP of	US 2001-343128P WO 2002-US41418	20011226 20021223
EP 1504017	A2	US 2004-845484 EP 2002-806227	20040512 20021223
JP 2005514032	W	WO 2002-US41418 WO 2002-US41418 JP 2003-558135	20021223 20021223 20021223

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002367391 EP 1504017	Al Based on A2 Based on	WO 2003057833 WO 2003057833
JP 2005514032	W Based on	WO 2003057833

PRIORITY APPLN. INFO: US 2001-343128P 20011226; US 2004-845484 20040512

- ANSWER 10 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN L2 Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent TImutant of a Chidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins.
- 2003-569236 [53] WPIDS AN
- WO2003054158 A UPAB: 20030820 AB

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host;
- (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell;
 - (5) a protein (VI) or its fragment encoded by (I);
 - (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and
 - (8) a kit comprising (I) or (II).
- USE (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed).

(I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used is assays to determine the phospholipid composition in biological membranes and as a fluorescent timer. Dwq.0/4

ACCESSION NUMBER:

2003-569236 [53] WPIDS

DOC. NO. CPI:

C2003-153632

TITLE:

Novel nucleic acid encoding a rapidly maturing chromo- or

fluorescent mutant of a Chidarian chromo- or fluorescent protein or its mutant, useful for

applications involving chromo- or fluorescent proteins.

43

DERWENT CLASS:

B04 D16

INVENTOR(S):
PATENT ASSIGNEE(S):

BEVIS, B; GLICK, B (UYCH-N) UNIV CHICAGO

W 20060119 (200606)

COUNTRY COUNT:

103

PATENT INFORMATION:

PATENT NO			KII	ND DATE				WEEK I			LA PG												
WO	2003	3054	1158	3	A2	200)301	703	(20	003	، (33	* El	 1	65	_								
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		ZM	ZW																				
AU	200	235	7322	2	A1	200	307	709	(20	0042	28)												
EP	145	5223	3		A2	200	0409	915	(20	046	50)	Eì	1										
	R:	AL	AT	BE	BG	CH	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	ΙE	ΙT	LI	LT	LU	LV	MC
		MK	NL	PT	RO	SE	SI	SK	TR														
US	200!	5149	9994	1	A1	200)507	707	(20	0054	17)												

APPLICATION DETAILS:

JP 2006501804

PATENT NO	KIND	APPLICATION	DATE			
WO 2003054158 AU 2002357322 EP 1456223	A2 A1 A2	WO 2002-US40539 AU 2002-357322 EP 2002-805620	20021218 20021218 20021218			
US 2005149994	Al Provisional CIP of	WO 2002-US40539 US 2001-341723P WO 2002-US40539	20021218 20011219 20021218			
JP 2006501804	W	US 2004-844064 WO 2002-US40539 JP 2003-554863	20040511 20021218 20021218			

FILING DETAILS:

PATENT NO	KIND	PATENT NO					
AU 2002357322	Al Based on	WO 2003054158					
EP 1456223	A2 Based on	WO 2003054158					
JP 2006501804	W Based on	WO 2003054158					

PRIORITY APPLN. INFO: US 2001-341723P 20011219; US 2004-844064 20040511

L2 ANSWER 11 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms.

AN 2003-156788 [15] WPIDS

AB WO 200296924 A UPAB: 20030303

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host;
- (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell;
 - (5) a protein (VI) or its fragment encoded by (I);
 - (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I);
- (8) a transgenic organism (IX) comprising a transgene that comprises (I);
 - (9) production of (VI);
- (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent;
- (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and
- (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).
- USE (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed).
- (I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

Dwg.0/10

ACCESSION NUMBER: 2

2003-156788 [15] WPIDS

DOC. NO. CPI:

C2003-040710

TITLE:

Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling

proteins, organelles, cells and organisms.

DERWENT CLASS:

B04 D16

INVENTOR (S):

CHUDAKOV, D; LUKYANOV, K; LUKYANOV, S A

PATENT ASSIGNEE(S):

(CHUD-I) CHUDAKOV D; (LUKY-I) LUKYANOV K; (LUKY-I)

LUKYANOV S A; (CLON-N) CLONTECH LAB INC

COUNTRY COUNT:

101

PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG

WO 2002096924 A1 20021205 (200315)* EN 96

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ

NL OA PT SD SE SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK

DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR

KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT

RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM '

ZW

US 2003092884 A1 20030515 (200335)

EP 1390379 A1 20040225 (200415) EN

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT

RO SE SI TR

AU 2002316164 A1 20021209 (200452)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE			
WO 2002096924	A1	WO 2002-US16379	20020524			
US 2003092884	A1 Provisional	US 2001-293752P	20010525			
	Provisional	US 2001-329176P	20011011			
		US 2002-155809	20020524			
EP 1390379	A1	EP 2002-746443	20020524			
		WO 2002-US16379	20020524			
AU 2002316164	A1	AU 2002-316164	20020524			

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 1390379	Al Based on	WO 2002096924
AU 2002316164	Al Based on	WO 2002096924

PRIORITY APPLN. INFO: US 2001-329176P

20011011; US

2001-293752P

20010525; US

2002-155809

20020524

=> d his

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(FILE 'HOME' ENTERED AT 11:08:06 ON 22 SEP 2006)

FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCAPLUS, WPIDS, FSTA, JICST-EPLUS, SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006

E LUKYANOV, S/AU

123 S CNIDARIAN AND (ANTHOZOAN)

L2 11 S L1 AND (NON-BIOLUMINESCENT)

=> s l1 and nucleic acid

4 FILES SEARCHED...

L4 16 L1 AND NUCLEIC ACID

=> d l14 ti abs ibib tot

L14 NOT FOUND

The L-number entered has not been defined in this session, or it has been deleted. To see the L-numbers currently defined in this session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> s 14 ti abs ibib tot
MISSING OPERATOR L4 TI

The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> d his

(FILE 'HOME' ENTERED AT 11:08:06 ON 22 SEP 2006)

FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCAPLUS, WPIDS, FSTA, JICST-EPLUS, SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006

E LUKYANOV, S/AU

L1 123 S CNIDARIAN AND (ANTHOZOAN)
L2 11 S L1 AND (NON-BIOLUMINESCENT)
L3 5 S L1 AND (NON-PENNATULACEAN)

L4 16 S L1 AND NUCLEIC ACID

=> d l4 ti abs ibib tot

L4 ANSWER 1 OF 16 MEDLINE on STN

TI The evolution of nuclear receptors: evidence from the coral Acropora.

We have amplified and sequenced PCR products derived from 10 nuclear receptor (NR) genes from the anthozoan cnidarian Acropora millepora, including five products corresponding to genes not previously reported from the phylum Cnidaria. cDNAs corresponding to seven of these products were sequenced and at least three encode full-length proteins, increasing the number of complete cnidarian NR coding sequences from one to four. All clear orthologs of Acropora NRs either lack an activation domain or lack a known ligand, consistent with the idea that the ancestral nuclear receptor was without a ligand. Phylogenetic analyses indicate that most, and possibly all, presently identified cnidarian NRs are members of NR subfamily 2, suggesting that the common ancestor of all known nuclear receptors most resembled members of this subfamily.

Copyright 2001 Academic Press.

ACCESSION NUMBER: 2001558273 MEDLINE DOCUMENT NUMBER: PubMed ID: 11603940

TITLE: The evolution of nuclear receptors: evidence from the coral

Acropora.

AUTHOR: Grasso L C; Hayward D C; Trueman J W; Hardie K M; Janssens

P A; Ball E E

CORPORATE SOURCE: Research School of Biological Sciences, Australian National

University, Canberra, ACT 2601, Australia.

SOURCE: Molecular phylogenetics and evolution, (2001 Oct) Vol. 21,

No. 1, pp. 93-102.

Journal code: 9304400. ISSN: 1055-7903.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200112

ENTRY DATE:

Entered STN: 18 Oct 2001

Last Updated on STN: 22 Jan 2002

Entered Medline: 5 Dec 2001

ANSWER 2 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN L4Novel nucleic acid encoding interconverted mutant of TIchromo-or fluorescent protein which are useful as biosensors, coloring agents;

involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic animal construction 2003-22525 BIOTECHDS

AN AB

DERWENT ABSTRACT:

NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromo- or fluorescent protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment of a nucleic acid encoding (I); (2) a construct comprising a vector and a nucleic acid encoding (I); (3) an expression cassette (II) comprises, a transcriptional initiation region that is functional in an expression host, a nucleic acid encoding (I) and a transcriptional termination region functional in the expression host; (4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell; (5) producing a chromo and/or fluorescent protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins; (6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III); (7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I); (8) a kit comprising a nucleic acid encoding (I); (9) preparation (M1) of nucleic acid encoding (I); and (10) a nucleic acid produced by (M1).

BIOTECHNOLOGY - Preparation: Producing a nucleic acid that encodes a protein having at least one point mutation chosen from positions 148 and 165 as compared to the parent protein produced by the nucleic acid encoding (I). The produced nucleic acid encodes a protein having point mutations at both positions 148 and 165. (I) is the fluorescent mutant of parent non-fluorescent chromoprotein. The nucleic acid produced encodes a protein further comprising mutations at positions 167 and 203, where (I) is a non-fluorescent chromoprotein of a parent fluorescent protein (claimed). Preferred Nucleic Acid : The chromo or fluorescent protein is from a non-bioluminescent Cnidarian sp and belongs to Anthozoan sp.. (I) includes a point mutation chosen from a mutation at positions 148 and 165. (I) is a fluorescent mutant of a chromoprotein and includes a point mutation at both positions 148 and 165. (I) is a non-fluorescent chromoprotein of a fluorescent protein and includes a point mutation at positions 167 and 203.

USE - Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. Chromoprotein encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The chromoprotein is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent

protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca2+ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

EXAMPLE - A purple chromoprotein, asCP from Anemonia sulcata and a red fluorescent protein DsRed from Discosoma Sp. were selected as representatives of chromoprotein (CP) and fluoroprotein (FP) respectively. Site directed and random mutagenesis were performed to transform CP into FP and vice versa. Site directed mutagenesis was performed by PCR with primers containing target substitution using the overlap extension method. All mutants were cloned into pQE30 vector so that recombinant proteins contained 6-histidine tag at their N-termini. Escherichia coli XL1 Blue cells were transformed with the plasmids according to standard protocols and spread onto 3-4 Petri dishes with LB agar media supplemented with ampicillin for selection. After overnight growth at 37degreesC the plates were stored for 2-5 days at room temperature or 4degreesC to allow proteins to mature completely. The plates were washed with phosphate buffered saline (PBS). Cells were disrupted by sonication and soluble recombinant proteins were purified on the TALON metal affinity resin. Absorption spectra of the proteins were recorded on a Beckman DU520 UV/VIS spectrophotometer. The amino acid substitution in asCP mutant in the positions 148 and 165, increased quantum yield of red fluorescence. Serine and valine were substituted at positions 148 and 165 respectively. In fluorescent DsRed, substitutions at position 148, 165, 167 and 203 significantly decreased fluorescence intensity, and the spectral characteristics of these mutants became more close to those of CPs. Non-fluorescent (NF) mutant DsRed-NF carried four amino acid substitutions, specifically. Ser148Cys, Ile165Asn, Lys167Met and Ser203Ala. DsRed-NF possessed a high extinction coefficient and an extremely low quantum yield (less than 0.001). These special characteristics converted DsRed-NF into a true chromoprotein. (56 pages)

ACCESSION NUMBER: 2003-22525 BIOTECHDS

TITLE: Novel nucleic acid encoding

interconverted mutant of chromo-or fluorescent protein which

are useful as biosensors; coloring agents;

involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic

animal construction

AUTHOR: BULINA M E; CHUDAKOV D; LUKYANOV K A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2003057833 17 Jul 2003 APPLICATION INFO: WO 2002-US41418 23 Dec 2002

PRIORITY INFO: US 2001-343128 26 Dec 2001; US 2001-343128 26 Dec 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-607998 [57]

L4 ANSWER 3 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving

chromo- or fluorescent proteins;

involving vector-mediated gene transfer and expression in Escherichia coli

2003-20948 BIOTECHDS

DERWENT ABSTRACT:

AN AB

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host; (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and (8) a kit comprising (I) or (II).

WIDER DISCLOSURE - (1) homologs of (I); (2) nucleic acids that encode proteins as (I), but differ in sequence of (I) due to degeneracy of genetic code; (3) nucleic acid that hybridize to (I); (4) nucleic acid that encode fusion proteins comprising (VI) and a heterologous protein; (5) homologs or fragments of (VI); and (6) polypeptides that differ from (VI).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) encodes a chromo- or fluorescent mutant of a non-bioluminescent Cnidarian species (e.g. Anthozoan species, preferably Discosoma sp.) chromo- or fluorescent protein. More preferably, (I) encodes a mutant of wild-type Discosoma sp. red fluorescent protein (DsRed), where the nucleic acid encodes a product having a point mutation at at least one of position 2, 5, 6, 21, 41, 42, 44 and 117 relative to wild-type DsRed, preferably a product having a point mutation at at least one of position 145 and 217. The nucleic acid encoding the product has a sequence of residues that is substantially the same or identical to a nucleotide sequence of at least 10 residues in length of a fully defined wild-type DsRed nucleic acid sequence (S1) as given in the specification.

USE - (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed). (I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used is assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

EXAMPLE - Wild-type Discosoma sp. red fluorescent protein (DsRed), an orange-red fluorescence with an emission maximum at 583 nm, had several problems for use as a fluorescent reporter, e.g., slow maturation. Therefore, to identify rapidly maturing DsRed variants, an earlier method for visualizing green fluorescent protein (GFP) fluorescence in microbial colonies was modified. Hexahistidine-tagged DsRed was produced at high levels in Escherichia coli. The fluorescence of the bacterial colonies was excited by placing a 520 +/- 20 nm bandpass filter over the lens of a slide projector, and the emission was detected. A library of mutant expression plasmids was generated using error-prone PCR to amplify the DsRed1 template. This library was transformed into E. coli, and over 100000 transformant colonies were examined. Colonies producing the wild-type DsRed1 protein required two days to develop significant fluorescence, but three mutant colonies showed strong fluorescence after one day of growth. Sequencing revealed that the three mutant plasmids were distinct, but that all of them contained an N42H codon change. Therefore a variant was generated that had only the N42H substitution. The N42H variant was purified in parallel with DsRed1, and the two proteins were analyzed by spectrofluorometry. The spectra of purified DsRed1 changed over a period of days as the protein matured. By contrast, the spectra of the purified N42H variant remained stable over time consistent with rapid maturation. In addition to accelerating maturation, the N42H substitution altered the spectral properties of the mature protein. Mature DsRed1 was assumed to be an equilibrium mixture of red fluorescent molecules and some green fluorescent molecules that were spectrally similar to GFP. The GFP-like species had a blue excitation peak at approximately 480 nm and a green emission peak at approximately 500 nm, but DsRed was tetramer, so excitation of the green molecules often resulted in the fluorescence resonance energy transfer (FRET) with neighboring red molecules to produce and emission. The FRET effect, together with the relatively low percentage of green molecules in mature DsRed1, yielded a very small peak of green emission relative to the red emission. In the N42H variant, the peaks of blue excitation and green emission were dramatically enhanced, indicating that the equilibrium had shifted so that a target percentage of the mature molecules contained the green chromophore. Because the N42H substitution considerably increased the size of the side chain, a more conservative N42Q substitution was also tried. This mutation required two base changes and probably would not have been present in the original mutant collection. The N42Q variant retained the rapid maturation property of the N42H variant, but showed much less blue excitation and green emission. The N42Q variant was therefore chosen as the starting point for further study. Additional mutagenesis yielded DsRed variants that showed even faster maturation and lower green emission than the original N42Q variant. After six rounds of mutagenesis, three optimized variants were selected and termed DsRed T1, DsRed T3 and DsRed T4. The spectral properties of DsRed T4 were virtually identical to those of DsRed Tl and very similar to those of the wild-type DsRed1. Compared with DsRed T1 and DsRed T4, DsRed T3 was somewhat brighter but has a significantly higher peak of blue excitation and a marginally higher peak of green emission. The optimized DsRed variants were examined both in vivo and in vitro. As judged by colony fluorescence, colony size, and plasmid stability, these variants were less toxic to E. coli than DsRedl, and they developed fluorescence more efficiently at growth temperatures of 37 degreesC and higher. Like wild-type DsRed, the optimized variants appeared to be tetrameric, they exhibited FRET between the green and red molecules. The end result was pair of optimized variants termed DsRed T3 and DsRed T4. DsRed T4, DsRed T3 matured rapidly, and the purified protein was nearly as bright as mature wild-type DsRed. Making this variant well suited to single-color imaging of red fluorescence. DsRed T3 had a higher peak of blue excitation and a slightly higher peak of green emission than wild-type DsRed. DsRed T4 had fluorescence spectra very similar to those of wild-type DsRed and yielded negligible contamination of the GFP signal.

Although purified DsRed T4 was only about half as bright as DsRed T3, this effect was partially offset in vivo because DsRed T4 matures nearly twice as fast as DsRed T3. Thus, DsRed T4 was probably the best variant for most applications. (65 pages)

ACCESSION NUMBER: 2003-20948 BIOTECHDS

Novel nucleic acid encoding a rapidly TITLE:

> maturing chromo- or fluorescent mutant of a Chidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins;

involving vector-mediated gene transfer and expression in

Escherichia coli

AUTHOR: BEVIS B; GLICK B

PATENT ASSIGNEE: UNIV CHICAGO

PATENT INFO: WO 2003054158 3 Jul 2003 APPLICATION INFO: WO 2002-US40539 18 Dec 2002

US 2001-341723 19 Dec 2001; US 2001-341723 19 Dec 2001 PRIORITY INFO:

DOCUMENT TYPE: Patent LANGUAGE: English

WPI: 2003-569236 [53] OTHER SOURCE:

ANSWER 4 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN L4

New nucleic acid encoding polypeptide products having TIat least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell

involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical and cosmetic industry 2003-15503 BIOTECHDS

AB DERWENT ABSTRACT:

lines;

AN

NOVELTY - A nucleic acid encoding a polypeptide product comprising a first and a second chromo/fluorescent domain, optionally joined by a linking domain, is new. The first and second chromo/fluorescent domains associate with each other under intracellular conditions so that the encoded polypeptide assumes a tertiary structure.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a construct comprising a vector and the above nucleic acid; (2) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the nucleic acid cited above;

(3) a cell, or its progeny, comprising an expression cassette cited above as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the cassette into the host cell; (4) producing the above polypeptide, comprising growing the cell cited above to express the polypeptide product; (5) a protein, or its fragment, encoded by the nucleic acid cited above; (6) an antibody binding specifically to the protein cited above; (7) a transgenic organism or transgenic cell or cell progeny, comprising a transgene that is the nucleic acid cited above; (8)

an application that employs a chromo- or fluorescent protein or a nucleic acid encoding the chromo- or fluorescent

protein, the improvement comprising employing the above protein or nucleic acid; and (9) a kit comprising the nucleic acid cited above.

BIOTECHNOLOGY - Preferred Nucleic Acid: The first and second chromo/fluorescent domains are oligomeric producing domains. The chromo/fluorescent domains are chromo- or fluorescent proteins from a Cnidarian species or mutants of chromo- or fluorescent proteins from a Chidarian species. The Cnidarian species is a non-bioluminiscent Cnidarian species, particularly an Anthozoan species. The nucleic acid encodes a fusion protein of the first and second chromo/fluorescent domains fused to a non-chromo/fluorescent protein domain.

USE - The nucleic acid and the protein are useful in producing labeled fusion proteins that have a precise and predictable signal to fusion partner ratio. The nucleic acid may also be used in generating transgenic, non-human plants or animals or site-specific gene modifications in cell lines. The chromoproteins may be used as coloring agents, as a food composition, in pharmaceuticals or cosmetics, as labels in analyte detection assays or as selectable markers in recombinant DNA applications. (34 pages)

ACCESSION NUMBER: 2003-15503 BIOTECHDS

TITLE: New nucleic acid encoding polypeptide

products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals

or site-specific gene modifications in cell lines;

involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical

and cosmetic industry

AUTHOR: LUKYANOV S A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2003031590 17 Apr 2003 APPLICATION INFO: WO 2002-US32560 10 Oct 2002

PRIORITY INFO: US 2002-383336 22 May 2002; US 2001-976673 12 Oct 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-381709 [36]

L4 ANSWER 5 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms;

vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

AN 2003-09318 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host; (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I); (8) a transgenic organism (IX) comprising a transgene that comprises (I); (9) production of (VI); (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent; (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

WIDER DISCLOSURE - Also disclosed are: (1) a composition comprising (I), (V), (VI), (VII) or recombinant vectors; (2) homologs of (I) or (VI); (3) nucleic acids that encode the proteins encoded by (I), but differ in sequence from (I) due to the degeneracy of the genetic code; (4) nucleic acids that hybridize to (I) under stringent conditions; (5)

nucleic acids encoding variants e.g., mutants of (I); and (6) nucleic acids that encode fusion proteins of (I) or its fragments.

BIOTECHNOLOGY - Preparation: (VI) is produced by growing (V), where (VI) is expressed, and isolating (VI) that is substantially free of other proteins (claimed). Preferred Nucleic Acid: In (I), the kindling stimulus is light of a kindling wavelength, intensity and duration effective to kindle the kindling fluorescent protein. The kindling wavelength of the kindling stimulus ranges from about 200-1500 nm, the kindling intensity of the kindling stimulus ranges from 0.01-106 W/cm2, and the kindling duration of the kindling stimulus ranges from 1 millisecond to about 60 minutes. The kindling fluorescent protein does not have a sequence that is identical to a sequence comprising 232 or 215 amino acids fully defined in the specification. The kindling fluorescent protein is a mutant of a wild type kindling fluorescent protein. The kindling fluorescent protein is a wild type protein or its mutant from a non-bioluminescent Cnidarian species, preferably an Anthozoan species. The second state is transient. The second fluorescent state is permanent. (I) is an isolated nucleic acid. Preferred Method: In (M), the kindling fluorescent protein is present inside an organism or cell.

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed). (I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

EXAMPLE - Generation of and initial characterization of kindling fluorescent proteins was as follows. Routine target i.e. site specific, and random mutagenesis of wild type asFP595 and Heteractis crispa chromoproteins was carried out. The asFP595 (asCP) had a sequence comprising 232 amino acids fully defined in the specification and was encoded by a nucleotide coding sequence comprising 767 base pairs fully defined in the specification. The H.crispa chromoprotein had a sequence comprising 215 amino acids fully defined in the specification was encoded by a nucleotide coding sequence comprising 760 base pairs fully defined in the specification. Mutagenesis was performed by the overlap extension method. Briefly, two overlapping fragments of each FP coding region were amplified. Polymerase chain reaction (PCR) was carried out using Advantage 2 polymerase mix in 1xmanufacturer's buffer supplemented with 100 muM of each dNTP, 0.2 muM of each primer, and 1 ng of plasmid DNA in 25 mul (final volume). To remove plasmids encoding wild type proteins, the 5' and 3'-fragments were excised from 2% low-melting agarose gel in 1xTAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. For each particular mutant, appropriate 5'- and 3'-fragments were combined to obtain full-length cDNA as follows. Equal volumes of 5'-fragment solution, 3'-fragment solution and 3xPCR mixture containing Advantage 2 polymerase mix, buffer and dNTPs were mixed together and subjected to 2-3 cycles of 95degreesC for 20 s, 65degreesC for 30 min, 72degreesC for 30 s. Then, the reaction was diluted 10 fold

and 1 mul of the diluted sample was used as a template for PCR with forward and reverse cloning primers. As a result, ready-for-cloning fragments containing full-length coding regions with target substitution(s) were generated. Mutant PC products were digested with endonucleases, for which the cloning primers contained sites, and then cloned into pQE30, digested with endonucleases generating complementary overhangs. Each of the recombinant proteins generated by both cloning-expression systems contained a 6xHis tag on the N- or C-terminus. Selected Escherichia coli clones were grown at 37degreesC in 50 ml to an optical density of (OD) 0.6. At that point, the expression of recombinant FP was induced with 0.2 mM isopropyl-beta-D-thiogalactoside (IPTG). The cultures were then incubated overnight. The following day, cells were harvested by centrifugation, resuspended in buffer and disrupted by sonication. Fluorescent proteins were purified from the soluble fraction. Proteins were at least 95% pure according to sodium dodecyl

sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (96 pages)

ACCESSION NUMBER: 2003-09318 BIOTECHDS

TITLE: Novel nucleic acid that is present in

other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g.

labeling proteins, organelles, cells and organisms;

vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer,

high throughput screening and cell sorting

AUTHOR: LUKYANOV S A; CHUDAKOV D; LUKYANOV K

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2002096924 5 Dec 2002 APPLICATION INFO: WO 2002-US16379 24 May 2002

PRIORITY INFO: US 2001-329176 11 Oct 2001; US 2001-293752 25 May 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-156788 [15]

L4 ANSWER 6 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

New nucleic acid encoding a non-aggregating chromoor fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant for analyte detection assays or fluorescence activated cell sorting applications;

vector-mediated reporter gene transfer and expression in host cell or transgenic animal for biosensor construction

AN 2003-03300 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid which encodes a

non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment of the novel nucleic acid; (2) a construct comprising a vector and the novel nucleic acid; (3) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the novel nucleic acid; (4) a cell, or its progeny, comprising the expression cassette of (3) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) producing a chromo- and/or fluorescent protein, comprising growing a cell of (4), where the protein is expressed, and isolating the protein free of other proteins; (6) a protein, or its fragment, encoded by the novel nucleic acid; (7) an antibody that binds specifically to the protein of (6); (8) a transgenic cell, or its progeny, or a transgenic organism comprising a transgene that is the above nucleic acid; (9) in an application that employs a chromo- or fluorescent protein, the improvement comprising employing the

protein of (6) or the novel nucleic acid; (10) a kit comprising the novel nucleic acid; and (11) producing the novel nucleic acid, comprising modulating at least one N-terminal residue codon of an aggregating Chidarian chromo- and/or fluorescent protein encoding sequence to produce the nucleic acid.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid has a sequence of residues that is substantially the same as or identical to at least 10 residues in length of a 678, 690, 707, 654 or 705 base pair sequence, given in the specification. The Chidarian chromo- or fluorescent protein is from a non-bioluminiscent Chidarian species, specifically an Anthozoan species. Preferred Method: In producing the novel nucleic acid, the residue is a basic residue, specifically, Lys or Arg. The modulation is a substitution of the basic residue for a neutral residue.

USE - The nucleic acid is useful in encoding Cnidarian chromo- or fluorescent protein useful in analyte detection assays, as coloring agents, as markers in recombinant DNA applications, as a sunscreen or filter, in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in screening assays, as second messenger detectors, in fluorescence activated cell sorting applications, in protease cleavage assays, or as a fluorescent timer. (80 pages)

ACCESSION NUMBER: 2003-03300 BIOTECHDS

TITLE: New nucleic acid encoding a

non-aggregating chromo- or fluorescent mutant of an

aggregating Cnidarian chromo- or fluorescent protein or mutant for analyte detection assays or fluorescence activated cell sorting applications;

vector-mediated reporter gene transfer and expression in host cell or transgenic animal for biosensor construction LUKYANOV S; LUKYANOV K; YANUSHEVICH Y; SAVISTKY A; FRADKOV A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2002068459 6 Sep 2002 APPLICATION INFO: WO 2002-US5749 20 Feb 2002

PRIORITY INFO: US 2001-6922 4 Dec 2001; US 2001-270983 21 Feb 2001

DOCUMENT TYPE: Patent LANGUAGE: English

AUTHOR:

OTHER SOURCE: WPI: 2002-691654 [74]

L4 ANSWER 7 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN

TI Rapidly maturing fluorescent proteins and methods for using the same

AB · Nucleic acid compns. encoding rapidly maturing

fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g.,

Anthozoan, species or are obtained from Anthozoan

non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. red fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety

of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER:

2005:588435 HCAPLUS

DOCUMENT NUMBER:

143:112117

TITLE:

Rapidly maturing fluorescent proteins and methods for

using the same

INVENTOR(S):

Bevis, Brooke; Glick, Benjamin The University of Chicago, USA

SOURCE:

U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of Appl.

No. PCT/US02/40539.

CODEN: USXXCO

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

PATENT ASSIGNEE(S):

PAT	PATENT NO.						KIND DATE			APPLICATION NO.					DATE			
	US 2005149994 WO 2003054158																	
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PRIORITY	APP	LN.	INFO	. :				US 2001-341723P				P 20011219						
									Ţ	WO 2	002-1	JS40!	539	7	A2 2	0021	218	

- ANSWER 8 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN L4
- Novel Cnidarian and Anthozoan chromo/fluoroproteins TIand cDNA encoding them
- DNA encoding novel chromo/fluoroproteins as well as the encoded proteins AB are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian , e.g., Anthozoan , species or are obtained from Anthozoan non- Pennatulacean (sea pen) species. proteins of interest include the following specific proteins: Heteractis crispa hcriGFP; Dendronephthya dendGFP; Zoanthus zoanRFP; Scolymia cubensis scubGFP1 and scubGFP2; Ricordea florida rfloRFP, rfloGFP2, and rfloGFP; Montastraea cavernosa mcavRFP, mcavGFP, and mcavGFP2; Condylactis gigantea cgigGFP; Agaricia fragilis afraGFP; and Montastraea annularis mannFP. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

2005:122690 HCAPLUS

ACCESSION NUMBER: DOCUMENT NUMBER:

142:193054

TITLE:

Novel Cnidarian and Anthozoan

chromo/fluoroproteins and cDNA encoding them Labas, Yulii Aleksandrovich; Gurskaya, Nadezda INVENTOR(S):

Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;

Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S):

Russia

U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of Appl. SOURCE:

No. PCT/02US/36499.

CODEN: USXXCO

DOCUMENT TYPE:

LANGUAGE:

Patent English

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

PAT	ENT	NO.			KIN	Ď	DATE		i	APPL:	I CAT	ION 1	NO.		D	ATE	
US	2005	0320	85		A1	_	2005	0210	1	US 2	004-	7573!	 56		2	0040	113
WO	2003	0424	01		A2		2003	0522	1	WO 2	002-1	US36	499		2	0021	112
WO	2003	0424	01		A3		2003	1120									
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PRIORITY	APP:	LN.	INFO	.:					Ī	JS 20	001-3	33298	80P		P 26	0011	113
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ANSWER 9 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN L4

Engineering rapidly maturing variants of the Discosoma red fluorescent TIprotein (DsRed) and their use as bioluminescent probes

Nucleic acid compns. encoding rapidly maturing AB

fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g.,

Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety

of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns.,

are provided. Claimed sequences were not present at the time of

publication.

ACCESSION NUMBER: 2003:511470 HCAPLUS

DOCUMENT NUMBER:

139:65739

TITLE:

Engineering rapidly maturing variants of the Discosoma

red fluorescent protein (DsRed) and their use as

bioluminescent probes

INVENTOR(S): PATENT ASSIGNEE(S):

Bevis, Brooke; Glick, Benjamin The University of Chicago, USA

PCT Int. Appl., 65 pp.

CODEN: PIXXD2

DOCUMENT TYPE:

Patent English

LANGUAGE:

SOURCE:

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

	PAT	FENT	NO.			KIN	D	DATE		1	APPL:	I CAT	ION	NO.		D	ATE	
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	EP	1456						2004										
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L4ANSWER 10 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN

cDNAs encoding chromo/fluoroproteins from non-bioluminescent TICnidarian species or non-Pennatulacean (sea pen) species and their use

Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded the same, are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Chidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. More specifically, they include GFP of Heteractis crispa, Dendronephthya sp, Scolymia cubensis, Ricordea florida, Montastraea cavernosa, Condylactis gigantea, Agaricia fragilis, sequence homolog of Montrastraea annularis and RFP of Zoanthus sp., Ricordea florida, and Montastraea cavernosa. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2003:397030 HCAPLUS

DOCUMENT NUMBER: 138:397335

AB

cDNAs encoding chromo/fluoroproteins from TITLE:

non-bioluminescent Cnidarian species or

non-Pennatulacean (sea pen) species and their use INVENTOR (S): Labas, Yulii Aleksandrovich; Gurskaya, Nadezda Georgievna; Yanushevich, Yuriy; Fradkov, Arcady

Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;

Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA

PCT Int. Appl., 88 pp. SOURCE:

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

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APPLICATION NO.
                                                                  DATE
    PATENT NO.
                        KIND
                               DATE
                               20030522 WO 2002-US36499
                                                                   20021112
                         A2
    WO 2003042401
                         A3
                                20031120
    WO 2003042401
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            PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT,
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            FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF,
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                               20030522
                                          CA 2002-2454031
                         AA
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    CA 2454031
                         A2
                                20040811
                                           EP 2002-797104
                                                                   20021112
    EP 1444245
        R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
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    JP 2005509420
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    US 2005032085
                                           US 2001-332980P P 20011113
WO 2002-US36499 W 20021112
PRIORITY APPLN. INFO.:
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L4 ANSWER 11 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN
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TI cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same Nucleic acid compns. encoding novel

Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded by the same, are provided. The subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific Anthozoan species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp. (NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red" (NFP-6), Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp. "magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2002:978391 HCAPLUS

DOCUMENT NUMBER: 138:50935

TITLE: cDNA and protein sequences of novel

chromo/fluoroproteins from non-bioluminescent

Cnidarian species or are obtained from

non-Pennatulacean (sea pen) species and methods for

using the same

INVENTOR(S): Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii

A.; Matz, Mikhail V.; Terskikh, Alexey

PATENT ASSIGNEE(S): Russia

SOURCE: U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of Appl.

No. PCT/US00/28477.

CODEN: USXXCO

DOCUMENT TYPE:

Patent English

LANGUAGE:

FAMILY ACC. NUM. COUNT:

17

PATENT INFORMATION:

PATEN	T NO.	KIND	DATE	APPLICATION NO.	DATE
WO 20	02197676 00034526 : JP	A1 A1		US 2001-6922 WO 1999-US29405	
		CY, DE	, DK, ES,	FI, FR, GB, GR, IE, I	IT, LU, MC, NL,
	-		20010419 20011206	WO 2000-US28477	20001013
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	34737	AA	•	ML, MR, NE, SN, TD, 7 CA 2002-2434737	
	02068459 02068459			WO 2002-US5749	20020220
	: AE, AG, AL, CO, CR, CU, GM, HR, HU, LS, LT, LU,	AM, AT, CZ, DE, ID, IL, LV, MA,	AU, AZ, DK, DM, IN, IS, MD, MG,	BA, BB, BG, BR, BY, EDZ, EC, EE, ES, FI, COJP, KE, KG, KP, KR, EM, MK, MN, MW, MX, MZ, MSI, SK, SL, TJ, TM, TSI, SK, SL, SL, SK, SL, SL, SK, SK, SL, SK, SK, SK, SK, SK, SK, SK, SK, SK, SK	GB, GD, GE, GH, KZ, LC, LK, LR, NO, NZ, OM, PH,
RI	UA, UG, US, W: GH, GM, KE, KG, KZ, MD,	UZ, VN LS, MW RU, TJ LU, MC	YU, ZA, MZ, SD, TM, AT, NL, PT,	ZM, ZW SL, SZ, TZ, UG, ZM, Z BE, CH, CY, DE, DK, E SE, TR, BF, BJ, CF, C	ZW, AM, AZ, BY, ES, FI, FR, GB,
	02254031 03022287	A1 A1	20020912	AU 2002-254031 US 2002-81864	
EP 13	85967 : AT, BE, CH,	A2 DE, DK	20040204 ES, FR,	EP 2002-723238 GB, GR, IT, LI, LU, N	
US 200 US 200		T2	20041209 20030515	JP 2002-567969 US 2002-155809 US 2005-187622	20050721
	PPLN. INFO.:	A1	20000330	US 1999-418529 US 1999-418917 US 1999-418922 US 1999-444338 US 1999-457556 US 1999-457898 US 1999-458144 US 1999-458477 WO 1999-US29405 US 2000-211607P US 2000-211609P US 2000-211626P US 2000-211627P US 2000-211687P US 2000-211766P US 2000-211880P	A2 19991014 B2 19991015 B2 19991015

US	2000-211888P	P	20000614
US	2000-212070P	P	20000614
WO	2000-US28477	A2	20001013
US	1998-210330	A	19981211
AU	2001-10867	A3	20001013
US	2001-270983P	P	20010221
US	2001-293752P	P	20010525
US	2001-329176P	P	20011011
US	2001-976673	A	20011012
US	2001-6922	A	20011204
US	2002-81864	A1	20020220
WO	2002-US5749	W	20020220

- L4 ANSWER 12 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.
- AN 2003-607998 [57] WPIDS
- AB WO2003057833 A UPAB: 20030906
 - NOVELTY Nucleic acid encoding an interconverted
 - mutant (I) of a chromo- or fluorescent protein, is new.
 - DETAILED DESCRIPTION INDEPENDENT CLAIMS are also included for:
 - (1) a fragment of a nucleic acid encoding (I);
 - (2) a construct comprising a vector and a nucleic
 acid encoding (I);
 - (3) an expression cassette (II) comprises, a transcriptional initiation region that is functional in an expression host, a nucleic acid encoding (I) and a transcriptional termination region functional in the expression host;
 - (4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell;
 - (5) producing a chromo and/or fluorescent protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins;
 - (6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III);
 - (7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I);
 - (8) a kit comprising a nucleic acid encoding (I);
 - (9) preparation (M1) of nucleic acid encoding
 (I); and
 - (10) a nucleic acid produced by (M1).
 - USE Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. Chromoprotein encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter é.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The chromoprotein is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca2+ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific

domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

DESCRIPTION OF DRAWING(S) - The figure shows the normalized spectra for selected mutants of asCP and DsRed.

Dwg.3/3

ACCESSION NUMBER:

2003-607998 [57] WPIDS

DOC. NO. CPI:

C2003-165725

TITLE:

Novel nucleic acid encoding

interconverted mutant of chromo-or fluorescent protein

which are useful as biosensors, coloring agents.

DERWENT CLASS:

B04 D16

INVENTOR (S):

BULINA, M E; CHUDAKOV, D; LUKYANOV, K A

PATENT ASSIGNEE(S):

(CLON-N) CLONTECH LAB INC; (BULI-I) BULINA M E; (CHUD-I)

38

CHUDAKOV D; (LUKY-I) LUKYANOV K A

COUNTRY COUNT:

103

JP 2005514032 W 20050519 (200538)

PATENT INFORMATION:

PA	rent	NO			KII	ND I	TAC	Ξ	V	VEE	ζ.		LA	1	PG								
WO	200	305	7833	3	A2	200	030	717	(20	0035	57) ¹	F E	.V	56	-								
	RW:	AT	BĒ	BG	CH	CY	CZ	DE	DK	ΕA	EE	ES	FI	FR	GB	GH	GM	GR	ΙE	IT	KE	LS	LU
		MC	MW	MZ	NL	OA	PΤ	SD	SĖ	SK	SL	SZ	TR	TZ	UG	ZM	ZW						
	W:	ΑE	AG	AL	AM	AT	AU	ΑZ	BA	BB	BG	BR	BY	ΒZ	CA	СН	CN	CO	CR	CU	CZ	DE	DK
		DM	DZ	EC	EE	ES	FI	GB	GD	GE	GH	GM	HR	HU	ID	IL	IN	IS	JP	KE	KG	KP	KR
		KZ	LC	LK	LR	LS	LT	LU	LV	MA	MD	MG	MK	MN	MW	MX	MZ	NO	NZ	OM	PH	PL	PT
		RO	RU	SC	SD	SE	SG	SK	SL	TJ	TM	TN	TR	TT	TZ	UA	UG	US	UZ	VC	VN	YU	ZA
		ZM	ZW																				
AU	2002	2367	7393	L	A1	200	030	724	(20	042	21)												
US	2004	1248	3180)	A1	200)412	209	(20	048	31)												
EP	1504	1017	7		A2	200	0502	209	(20	051	L2)	Eì	J										
	R:	AL	AT	BE	BG	CH	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	ΙE	IT	LI	LT	LU	LV	MC
		MK	NL	PТ	RO	SE	ST	SK	TR														

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003057833 AU 2002367391 US 2004248180	A2 A1 A1 Provisional CIP of	WO 2002-US41418 AU 2002-367391 US 2001-343128P WO 2002-US41418	20021223 20021223 20011226 20021223
EP 1504017	A2	US 2004-845484 EP 2002-806227 WO 2002-US41418	20040512 20021223 20021223
JP 2005514032	W	WO 2002-US41418 JP 2003-558135	20021223 20021223

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002367391	Al Based on	WO 2003057833
EP 1504017	A2 Based on	WO 2003057833
JP 2005514032	W Based on	WO 2003057833

PRIORITY APPLN. INFO: US 2001-343128P 20011226; US

L4 ANSWER 13 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

TI Novel nucleic acid encoding a rapidly maturing chromoor fluorescent mutant of a Chidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins.

AN 2003-569236 [53] WPIDS

AB WO2003054158 A UPAB: 20030820

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or fluorescent mutant of a Chidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a fragment (II) of (I);

(2) a construct (III) comprising a vector and (I);

- (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host;
- (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell;
 - (5) a protein (VI) or its fragment encoded by (I);
 - (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and
 - (8) a kit comprising (I) or (II).
- USE (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed).
- (I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used is assays to determine the phospholipid composition in biological membranes and as a fluorescent timer. Dwg.0/4

ACCESSION NUMBER: 2003-569236 [53] WPIDS

DOC. NO. CPI: C2003-153632

TITLE: Novel nucleic acid encoding a rapidly

maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or

fluorescent proteins.

DERWENT CLASS: B04 D16

INVENTOR(S): BEVIS, B; GLICK, B
PATENT ASSIGNEE(S): (UYCH-N) UNIV CHICAGO

COUNTRY COUNT: 103

PATENT INFORMATION:

PAT	rent	NO		. :	KI	ND I	TAC	Ξ		WEE!	K		LA]	PG								
WO	2003	3054	4158	3	A2	200	0307	703	(20	003!	53)	* Ei	J	65									
	RW:	AT	BE	BG	CH	CY	CZ	DE	DK	EA	EE	ES	FI	FR	GB	GH	GM	GR	ΙE	ΙT	KE	LS	LU
		MC	MW	MZ	NL	OA	PT	SD	SE	SI	SK	SL	SZ	TR	TZ	UG	ZM	ZW					
	W:	AE	AG	AL	AM	AT	AU	AZ	BA	BB	BG	BR	BY	ΒZ	CA	CH	CN	CO	CR	CU	CZ	DE	DK
		DM	DZ	EC	EE	ES.	FI	GB	GD	GE	GH	GM	HR	HU	ID	IL	IN	IS	JP	KE	KG	KP	KR
		KZ	LC	LK	LR	LS	LT	LU	LV	MA	MD	MG	MK	MN	MW	MX	MZ	NO	NZ	OM	PH	PL	PT
		RO	RU	SC	SD	SE	SG	SK	SL	TJ	TM	TN	TR	TT	TZ	UA	UG	US	UZ	VC	VN	YU	ZA
		ZM	ZW																				
AU	2002	235	7322	2	A1	200	030	709	(20	0042	28)												
ΕP	1456	5223	3		A2	200	0409	915	(20	004	50)	Eì	1										
	R:	AL	AT	BE	BG	CH	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	ΙE	IT	LI	LT	LU	LV	MC
	·	MK	NL	PT	RO	SE	SI	SK	TR														

· 43

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003054158	A2	WO 2002-US40539	20021218
AU 2002357322	A1	AU 2002-357322	20021218
EP 1456223	A2	EP 2002-805620	20021218
		WO 2002-US40539	20021218
US 2005149994	Al Provisional	US 2001-341723P	20011219
	CIP of	WO 2002-US40539	20021218
		US 2004-844064	20040511
JP 2006501804	W	WO 2002-US40539	20021218
		JP 2003-554863	20021218

FILING DETAILS:

AU 2002357322 Al Based on WO 2003054158	
EP 1456223 A2 Based on WO 2003054158 JP 2006501804 W Based on WO 2003054158	8

PRIORITY APPLN. INFO: US 2001-341723P 20011219; US 2004-844064 20040511

US 2005149994 A1 20050707 (200547) JP 2006501804 W 20060119 (200606)

- L4 ANSWER 14 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines.
- AN 2003-381709 [36] WPIDS
- CR 2002-444170 [47]
- AB WO2003031590 A UPAB: 20050411

NOVELTY - A nucleic acid encoding a polypeptide product comprising a first and a second chromo/fluorescent domain, optionally joined by a linking domain, is new. The first and second chromo/fluorescent domains associate with each other under intracellular conditions so that the encoded polypeptide assumes a tertiary structure.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

- (1) a construct comprising a vector and the above nucleic acid;
- (2) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the nucleic acid cited above;

- (3) a cell, or its progeny, comprising an expression cassette cited above as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the cassette into the host cell;
- (4) producing the above polypeptide, comprising growing the cell cited above to express the polypeptide product;
- (5) a protein, or its fragment, encoded by the nucleic acid cited above;
 - (6) an antibody binding specifically to the protein cited above;
- (7) a transgenic organism or transgenic cell or cell progeny, comprising a transgene that is the nucleic acid cited above:
- (8) an application that employs a chromo- or fluorescent protein or a nucleic acid encoding the chromo- or fluorescent protein, the improvement comprising employing the above protein or nucleic acid; and
- (9) a kit comprising the nucleic acid cited above.

USE - The nucleic acid and the protein are useful in producing labeled fusion proteins that have a precise and predictable signal to fusion partner ratio. The nucleic acid may also be used in generating transgenic, non-human plants or animals or site-specific gene modifications in cell lines. The chromoproteins may be used as coloring agents, as a food composition, in pharmaceuticals or cosmetics, as labels in analyte detection assays or as selectable markers in recombinant DNA applications.

Dwg.0/7

ACCESSION NUMBER:

2003-381709 [36] WPIDS

CROSS REFERENCE:

2002-444170 [47]

DOC. NO. CPI:

C2003-101460

TITLE:

New nucleic acid encoding polypeptide

products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell

lines.

DERWENT CLASS:

B04 D13 D16

INVENTOR (S):

LUKYANOV, S A

PATENT ASSIGNEE(S):

(CLON-N) CLONTECH LAB INC

COUNTRY COUNT:

102

PATENT INFORMATION:

PAT	FENT	NO			KIN	ND I	DATI	Ξ 	V 	VEE	Χ 		LA	I 	PG								
WO	200	303:	1590)	A2	200	0304	117	(20	0033	36) 1	EI	1	34									
	RW:	AT	BE	BG	CH	CY	CZ	DE	DK	EA	EE	ES	FI	FR	GB	GH	GM	GR	ΙE	ΙT	KE	LS	LU
		MC	MW	MZ	NL	OA	PT	SD	SE	SK	SL	SZ	TR	TZ	UG	ZM	ZW						
	W:	ΑE	AG	AL	AM	AT	AU	ΑZ	BA	BB	BG	BR	BY	BZ	CA	CH	CN	CO	CR	CU	CZ	DE	DK
		DM	DZ	EC	EE	ES	FI	GB	GD	GE	GH	GM	HR	HU	ID	IL	IN	IS	JP	KE	KG	ΚP	KR
		KZ	LC	LK	LR	LS	LT	LU	LV	MA	MD	MG	MK	MN	MW	MX	MZ	ИО	NZ	OM	PH	PL	PT
		RO	RU	SD	SE	SG	SI	SK	SL	TJ	TM	TN	TR	TT	ΤŹ	UA	UG	US	UZ	VC	VN	YU	ZA
		ZM	ZW																				
EP	143	4483	3		A2	200	0407	707	(20	044	14)	E	1										
	R:	AL	AT	BE	BG	CH	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	ΙE	IT	LI	LT	LU	LV	MC
		MK	NL	PT	RO	SE	SI	SK	TR														
AU	200	2362	2771	L	A1	200	304	122	(20	046	50)												
US	200	4216	5180)	A1	200)41(28	(20	0047	71)												
JP	200	550′	7657	7	W	200)503	324	(20	052	23)]	105									

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003031590	A2	WO 2002-US32560	20021010

I	EΡ	1434483	A2		EP	2002-801039	20021010
					WO	2002-US32560	20021010
1	UA	2002362771	A1		AU	2002-362771	20021010
Į	US	2004216180	A1	CIP of	US	2001-976673	20011012
				Provisional	US	2002-356225P	20020211
				Provisional	US	2002-383336P	20020522
				CIP of	WO	2002-US32560	20021010
					US	2004-806930	20040322
Ĺ	JP	2005507657	W		WO	2002-US32560	20021010
					JΡ	2003-534561	20021010

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 1434483 AU 2002362771 JP 2005507657	A2 Based on A1 Based on W Based on	WO 2003031590 WO 2003031590 WO 2003031590
PRIORITY APPLN. INFO	: US 2002-383336P 2001-976673 2002-356225P 2004-806930	20020522; US 20011012; US 20020211; US 20040322

- L4 ANSWER 15 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms.
- AN 2003-156788 [15] WPIDS
- AB WO 200296924 A UPAB: 20030303

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host;
- (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell;
 - (5) a protein (VI) or its fragment encoded by (I);
 - (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I);
- (8) a transgenic organism (IX) comprising a transgene that comprises (I);
 - (9) production of (VI);
- (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent;
- (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and
- (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).
- USE (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein

label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed).

(I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

Dwg.0/10

ACCESSION NUMBER:

2003-156788 [15] WPIDS

DOC. NO. CPI:

C2003-040710

TITLE:

Novel nucleic acid that is present in

other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and

organisms.

DERWENT CLASS:

B04 D16

INVENTOR (S):

CHUDAKOV, D; LUKYANOV, K; LUKYANOV, S A

PATENT ASSIGNEE(S):

(CHUD-I) CHUDAKOV D; (LUKY-I) LUKYANOV K; (LUKY-I)

LUKYANOV S A; (CLON-N) CLONTECH LAB INC

COUNTRY COUNT:

101

PATENT INFORMATION:

PATENT NO	KIND DATE	WEEK	LA	PG

WO 2002096924 A1 20021205 (200315) * EN 96

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

US 2003092884 A1 20030515 (200335) EP 1390379 A1 20040225 (200415) EN

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR

AU 2002316164 A1 20021209 (200452)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE	
WO 2002096924	A1	WO 2002-US16379	20020524	
US 2003092884	Al Provisional	US 2001-293752P	20010525	
	Provisional	US 2001-329176P	20011011	
		US 2002-155809	20020524	
EP 1390379	A1	EP 2002-746443	20020524	
		WO 2002-US16379	20020524	
AU 2002316164	A1	AU 2002-316164	20020524	

FILING DETAILS:

PATENT I	NO	KIND	PATENT NO	
				_

EP 1390379 Al Based on WO 2002096924 AU 2002316164 Al Based on WO 2002096924

PRIORITY APPLN. INFO: US 2001-329176P 20011011; US 2001-293752P 20010525; US 2002-155809 20020524

L4 ANSWER 16 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

TI New nucleic acid encoding a non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant for analyte detection assays or fluorescence activated cell sorting applications.

AN 2002-691654 [74] WPIDS

CR 2000-423373 [36]; 2000-423374 [36]; 2000-423375 [36]; 2000-423376 [36]; 2000-423377 [36]; 2000-423378 [36]; 2000-423379 [36]; 2000-423380 [36]; 2000-423381 [36]; 2000-423451 [36]; 2001-266409 [27]; 2002-154595 [20]

AB WO 200268459 A UPAB: 20060302

NOVELTY - A nucleic acid which encodes a

non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a fragment of the novel nucleic acid;
- (2) a construct comprising a vector and the novel nucleic acid;
- (3) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the novel nucleic acid;
- (4) a cell, or its progeny, comprising the expression cassette of (3) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell;
- (5) producing a chromo- and/or fluorescent protein, comprising growing a cell of (4), where the protein is expressed, and isolating the protein free of other proteins;
- (6) a protein, or its fragment, encoded by the novel nucleic acid;
 - (7) an antibody that binds specifically to the protein of (6);
- (8) a transgenic cell, or its progeny, or a transgenic organism comprising a transgene that is the above nucleic acid;
- (9) in an application that employs a chromo- or fluorescent protein, the improvement comprising employing the protein of (6) or the novel nucleic acid;
 - (10) a kit comprising the novel nucleic acid; and
- (11) producing the novel nucleic acid, comprising modulating at least one N-terminal residue codon of an aggregating Cnidarian chromo- and/or fluorescent protein encoding sequence to produce the nucleic acid.
- USE The nucleic acid is useful in encoding Cnidarian chromo- or fluorescent protein useful in analyte detection assays, as coloring agents, as markers in recombinant DNA applications, as a sunscreen or filter, in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in screening assays, as second messenger detectors, in fluorescence activated cell sorting applications, in protease cleavage assays, or as a fluorescent timer.

Dwg.0/16

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ACCESSION NUMBER: 2002-691654 [74] WPIDS

CROSS REFERENCE: 2000-423373 [36]; 2000-423374 [36]; 2000-423375 [36]; 2000-423377 [36]; 2000-423378 [36]; 2000-423379 [36]; 2000-423380 [36]; 2000-423381 [36]; 2000-423451 [36]; 2001-266409 [27]; 2002-154595 [20]

DOC. NO. CPI: C2002-195501
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TITLE: New nucleic acid encoding a

non-aggregating chromo- or fluorescent mutant of an

aggregating Cnidarian chromo- or fluorescent

protein or mutant for analyte detection assays or fluorescence activated cell sorting applications.

DERWENT CLASS: B04 D16 D21 D22

INVENTOR(S): FRADKOV, A F; LABAS, Y A; LUKYANOV, S A; MATZ, M V;

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SAVITSKY, A; YANUSHEVICH, Y; SAVISTKY, A

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LUKYANOV S; (SAVI-I) SAVITSKY A; (YANU-I) YANUSHEVICH Y;

(CLON-N) CLONTECH LAB INC

COUNTRY COUNT: 101

PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG

WO 2002068459 A2 20020906 (200274)* EN 80

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ

NL OA PT SD SE SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR

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RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

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AU 2002254031 A8 20051020 (200615)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
PATENT NO	A2 A1 CIP of Provisional	WO 2002-US5749 US 1999-418529 US 1999-418917 US 1999-444338 US 1999-444341 US 1999-457556 US 1999-457898 US 1999-458144 US 1999-458477 US 2000-211607P US 2000-211609P US 2000-211626P US 2000-211687P US 2000-211687P US 2000-211687P US 2000-211880P US 2000-211880P US 2000-211888P US 2000-212070P	20020220 19991014 19991015 19991015 19991119 19991209 19991209 19991209 19991209 20000614 20000614 20000614 20000614 20000614 20000614 20000614 20000614
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				WO	2002-US5749	20020220
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				US	2005-187622	20050721
AU	2002254031	A8		AU	2002-254031	20020220

FILING DETAILS:

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	AU	2002254031	A8	Based on	WO	2002068459		

PRIORITY APPLN. INFO: US 2001-6922 20011204; US 2001-270983P 20010221; WO 1999-US29405 19991210; US 2002-81864 20020220; US 2005-187622 20050721